UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

030801

NOV 14 1995

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **2,4-DB.** Metabolism Committee Question Re: Regulation of Metabolites in Plant and Animal Commodities.
DP Barcode: D220888; CBRS No.: 16473; No MRID No., Rereg. Case No.: 0196.

FROM: David J. Miller, HSO, US Public Health Service
Chemistry Pilot Review Team
Chemistry Branch II--Reregistration Support
Health Effects Division (7509C)

THRU: Edward Zager, Branch Chief
Chemistry Branch II--Reregistration Support
Health Effects Division (7509C)

TO: Metabolism Committee
Health Effects Division (7509C)

The compound 2,4-DB (2,4-dichlorophenoxybutyric acid) is a phenoxyalkanoic acid-type herbicide. It is used to control broadleaf weeds in soybeans, peanuts, seedling and established alfalfa, seedling birdsfoot trefoil, and seedling clover. It is generally agreed that 2,4-DB (and other straight-chain, even-numbered higher analogues) are toxic only to plants that possess an active beta-oxidation enzyme system capable of degrading the herbicide to the growth regulator compound 2,4-D; it is the 2,4-D degradate that accumulates in regions of active metabolism and causes cell division, cell enlargement, and (if present in sufficient concentrations) cell death.

Tolerances (40 CFR 180.331) exist for the combined residues of 2,4-DB and its 2,4-D metabolite in/on alfalfa, clover, mint, peanuts, soybean, and birdsfoot trefoil. There are presently no tolerances on meat, milk, poultry, and egg commodities. There are no established or proposed CODEX MRLs for this pesticide and thus there are no issues of compatability with respect to U.S. tolerances and CODEX MRLs.

The Pesticide Analytical Manual (Vol II) lists as Method I a GC Method with microcoulometric detection for the enforcement of tolerances for 2,4-DB residues; this is the same as the PAM Vol I multiresidue method for detection of chlorphenoxy acid residues in food.

The 2,4-DB Reregistration Standard (2/1/88) concluded that the qualitative nature of the residue of 2,4-DB in animals and plants was not adequately understood, and required that data be submitted depicting the metabolism of ring-labeled [^{14}C]2,4-DB in plants and animals.

In response, the 2,4-DB Task Force submitted data in 1993 on metabolism in alfalfa, peanuts, soybeans, laying hens, and lactating goats. CBRS has reviewed this information and concluded that the nature of the residue in plants and animals is understood (D. Miller, CBRS Nos. 12753, 12931, and 12963, currently under review).

The HED Metabolism Committee has previously reviewed similar questions with respect to the herbicide 2,4-D *per se* as well as with the herbicide MCPB (2-methyl-4-chlorophenoxy butyric acid), a ring-substituted analogue of 2,4-DB. In the former case, the Metabolism Committee concluded that the residue to be regulated in wheat and similar crops and well as in animal products was parent 2,4-D *per se*, while in the latter case, the Committee concluded that the residues of concern for both tolerance and risk assessment are both parent MCPB and its 4-chloro-2-methyl phenoxy acetic acid metabolite; the Committee concluded that an identified 2-hydroxymethyl-substituted metabolite (CHTA) was not of concern and should not be included in the tolerance expression.

The structure of 2,4-DB and its various metabolites are presented in Table 1 along with the plant and animal matrices in which each respective metabolite was identified.

QUESTIONS TO THE COMMITTEE:

1. In plant commodities, which residues of 2,4-DB should be regulated?

2. In ruminant and poultry commodities, which residues of 2,4-DB should be regulated?

DETAILED ANALYSIS:

This review is divided into two parts: the first part reviews the results of the plant metabolism studies in alfalfa, peanuts, and soybean. The second part reviews the results of the ruminant and poultry metabolism studies. The Committee is being asked to determine which 2,4-DB metabolites should be regulated in plant and animal commodities.

Plant Metabolism Studies

The nature of the 2,4-DB residue in plants is reviewed in detail in a previous CBRS Review (D. Miller, CBRS Nos 12753, 12931, and 12963, currently under review). This memorandum concluded the nature of 2,4-DB residue in plants is adequately understood based on acceptable plant metabolism studies in alfalfa, peanuts, and soybeans conducted at a 1.1x application rate. This information is reviewed briefly below for the tested crops:

Alfalfa: Total radioactive residues (expressed as 2,4-DB equivalents) were 4.12 ppm in 59-day PHI forage, and 16.42 ppm in 59-day PHI hay harvested from outdoor-grown alfalfa plants treated with a single foliar broadcast application of [^{14}C]2,4-DB at 1.1x the maximum use rate. Detailed results of these trials are presented in Table 2. Briefly, a total of 80-100% of the radioactive residue found in forage and hay was identified: unchanged 2,4-DB parent was the major residue in both forage and hay, with the parent comprising approximately one-half of the TRR in 59-day forage and in 59-day PHI hay (2.34 ppm and 7.96 ppm, respectively). Other significant residues in alfalfa forage and hay included 4-chlorophenoxyacetic acid (at ca. 8% TRR) and various dichlorohydroxyphenoxy-acetic and butyric acids.

Based on the evidence presented, it is proposed that 2,4-DB is degraded in alfalfa to form 2,4-D, 4-CPA, and other phenoxyacetic- and phenoxybutyric- acid metabolites. The presence of 2,5-dichloro-4-hydroxybutyric acid (and the potential presence of 3,5-dichloro-2-hydroxyphenoxy butyric acid) indicates a position shift of the chlorine atom. Side chain degradation, ring hydroxylation, and chlorine shift are the major metabolic pathways for 2,4-DB in alfalfa.

Peanuts Total radioactive residues were 43.77 ppm in forage, 238.34 ppm in hay, 0.16 ppm in seed, 0.10 ppm in hulls, and 3.62 ppm in vines from outdoor-grown peanut plants harvested 0 days (forage and hay) and 148 days (seed, hulls, and vines) following the second of two foliar treatments of [^{14}C]2,4-DB applied at 1.1x the maximum seasonal application rate. Detailed results from these trials are presented in Table 3. Briefly, approximately 35-80% of the TRR was identified with parent 2,4-DB present as the major metabolite (>50% TRR) in 0-day forage and hay, but detected as only a minor residue in vines (2,4-DB parent was not detected in seed and hulls and no non-zero day PHI data are available for peanut forage or hay). In vines, seed, and hulls, the major metabolites were various hydroxy-substituted dichlorophenoxyacetic acids and dichlorophenoxyy phenols. The 2,4-D metabolite was detected only in forage and hay at 2-3% TRR. Other major residues identified in peanuts were hydroxy-substituted 2,4-D in forage, hay, and seeds, chlorohydroquinone in hulls, and 2,4-dichloro-6-hydroxyphenoxyacetic acid in vines. Fractionation of non-extractable residues indicated that some radioactivity was incorporated into natural constituents (sugars, organic acids, starch, protein, pectin, lignin, hemicellulose,

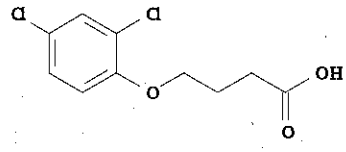
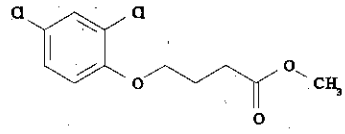
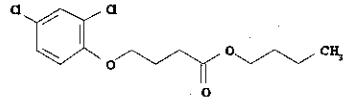
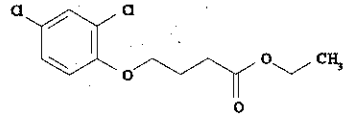
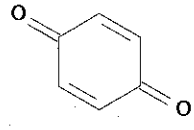
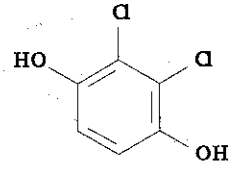
and cellulose).

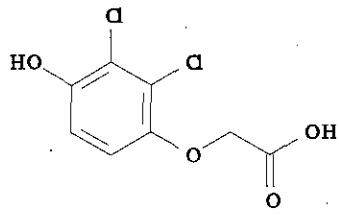
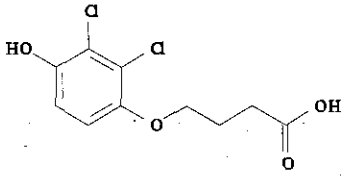
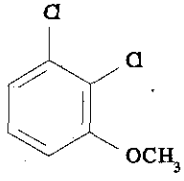
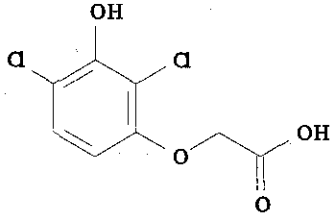
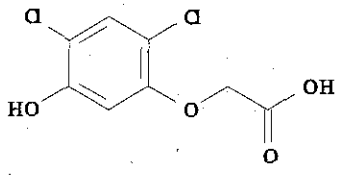
Based on the evidence presented, it is proposed that 2,4-DB is metabolized by side chain degradation to form 2,4-D in peanuts. The presence of 4-CPA and other dechlorinated metabolites indicates that some dechlorination occurs. The presence of 2,3-Cl-4-HOH at significant levels in a variety of matrices indicates a position shift of the chlorine atom. Side chain degradation, ring hydroxylation, and chlorine shift are the major metabolic pathways for 2,4-DB in peanut plants.

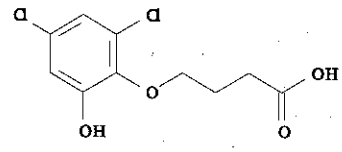
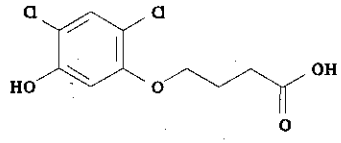
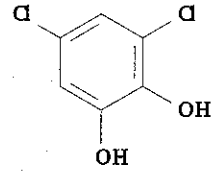
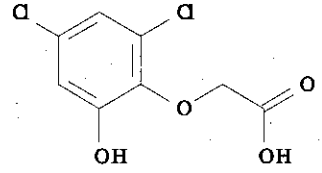
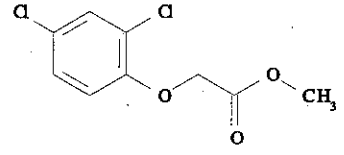
Soybeans Total radioactive residues were 5.33 ppm in forage and 20.76 ppm in hay harvested 34 days, and 0.07 ppm in seed, 0.19 ppm in pods, and 5.58 ppm in vines harvested 84 days following treatment of outdoor-grown soybean plants with one foliar broadcast application of [^{14}C]2,4-DB applied at a 1.1x application rate). Detailed results from these trials are presented in Table 4. Briefly, approximately 20-70% of the radioactive residue found in forage, hay, seeds, pods, and vines was identified. Parent 2,4-DB was found in all soybean matrices except seed and was the major residue in forage and hay accounting for 10-20% of TRR. The 2,4-D metabolite was detected at minor levels in forage, hay, and vines; 1,4-benzoquinone was the major metabolite in seeds, pods, and vines (accounting for up to ca. 25% TRR, or 1.62 ppm), and was also detected in forage and hay. A variety of hydroxy-substituted 2,3- and 2,4-D metabolites were seen in many of the commodities. Fractionation of the non-extractable residues revealed some incorporation into natural plant products.

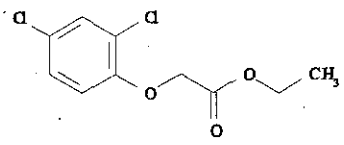
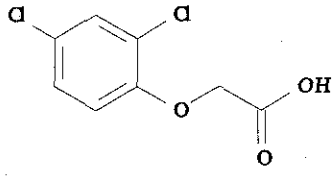
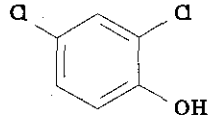
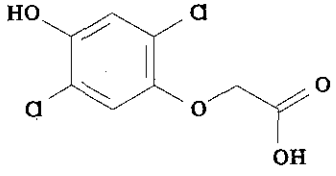
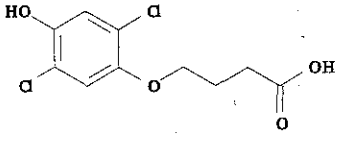
Based on the evidence presented, it is proposed that 2,4-DB is metabolized by side chain degradation and ring hydroxylation to form 2,4-D and various hydroxylated derivatives. The presence of 2,3- and 2,5- chlorinated derivatives at significant levels indicates that a position shift of the chlorine atom also occurs. Side chain degradation, ring hydroxylation, and chlorine shift are the major pathways for 2,4-DB in soybean matrices.

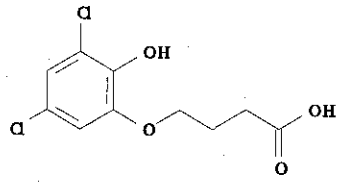
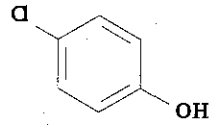
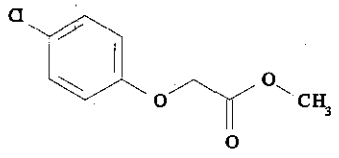
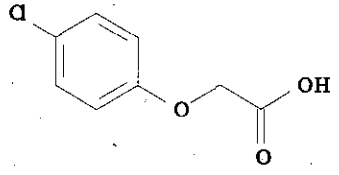
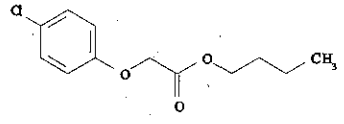
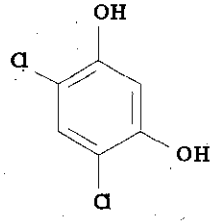
Table 1. 2,4-DB and its metabolites in plants and animals (MRIDs 42965901, 43009801, 43033802, 43033803, and 43033901).

Common Name Chemical Name	Structure	Substrate
2,4-DB (4-(2,4- dichlorophenoxy)-butyric acid		alfalfa forage and hay peanut forage, hay, and vines soybean forage, hay, seed, pods, and vines hen liver, fat, and egg yolk goat liver and kidney
2,4-DB methyl ester (4-(2,4-dichlorophenoxy)butyric acid, methyl ester		alfalfa forage peanut forage and hay
2,4-DB butyl ester (4-(2,4-dichlorophenoxy)butyric acid, butyl ester		peanut forage and hay soybean hay, seed, and pods
2,4-DB ethyl ester (4-(2,4-dichlorophenoxy)butyric acid, ethyl ester		peanut vines
1,4-BQ 1,4-benzoquinone		soybean forage, hay, seed, pods, and vines
2,3-Cl-4-HOH 2,3-dichloro-4-hydroxyphenol		peanut forage, hay, and vines soybean hay and vines

Common Name Chemical Name	Structure	Substrate
2,3-Cl-4-HPAA 2,3-dichloro-4-hydroxyphenoxyacetic acid		peanut forage, seed, hulls, and vines soybean forage and vines
2,3-Cl-4-HPBA 2,3-dichloro-4-hydroxyphenoxybutyric acid		peanut forage soybean hay and vines
2,3-DCA 2,3-dichloroanisole		soybean forage, hay and vines
2,4-Cl-3-HPAA 2,4-dichloro-3-hydroxyphenoxyacetic acid		peanut forage, hay, and vines soybean forage, hay, and vines
2,4-Cl-5-HPAA 2,4-dichloro-5-hydroxyphenoxyacetic acid		peanut forage and vines soybean hay and vines

Common Name Chemical Name	Structure	Substrate
2,4-Cl-6-HPBA 2,4-dichloro-6-hydroxyphenoxybutyric acid		alfalfa hay peanut vines soybean vines
2,4-Cl-5-HPBA 2,4-dichloro-5-hydroxyphenoxybutyric acid		peanut hay and vines soybean forage and vines
2,4-Cl-6-HOH 2,4-dichloro-6-hydroxyphenol		peanut vines soybean hay and vines
2,4-Cl-6-HPAA 2,4-dichloro-6-hydroxyphenoxyacetic acid		alfalfa hay peanut forage, hay, seed, and vines soybean forage, hay, pods, and vines
2,4-D methyl ester 2,4-dichlorophenoxyacetic acid, methyl ester		soybean vines

Common Name Chemical Name	*Structure*	*Substrate*
2,4-D ethyl ester 2,4-dichlorophenoxyacetic acid, ethyl ester		soybean hay and vines
2,4-D 2,4-dichlorophenoxyacetic acid		alfalfa forage and hay peanut forage and hay soybean forage, hay, and vines hen liver goat liver and kidney
2,4-D phenol 2,4-dichlorophenol		alfalfa forage and hay peanut hay soybean forage and hay hen liver goat liver and kidney
2,5-Cl-4-HPAA 2,5-dichloro-4-hydroxyphenoxyacetic acid		alfalfa hay soybean forage, pods, and vines
2,5-Cl-4-HPBA 2,5-dichloro-4-hydroxyphenoxybutyric acid		alfalfa forage and hay peanut forage and hay

Common Name Chemical Name	Structure	Substrate
3,5-Cl-2-HPBA 3,5-dichloro-2-hydroxyphenoxybutyric acid		alfalfa forage soybean forage
4-CP 4-chlorophenol		alfalfa hay soybean hay
4-CPA methyl ester 4-chlorophenoxyacetic acid, methyl ester		soybean forage and hay
4-CPA 4-chlorophenoxyacetic acid		alfalfa forage and hay peanut forage, hay, seed, and vines soybean forage, hay and vines
4-CPA butyl ester 4-chlorophenoxyacetic acid, butyl ester		peanut hay and vines soybean forage, hay, and vines
4,6-DCR 4,6-dichlororesorcinol		alfalfa forage peanut hay soybean forage, hay, and vines

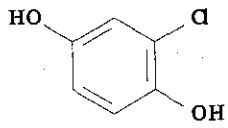
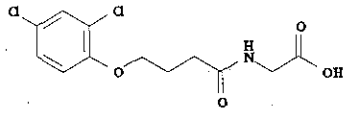
Common Name Chemical Name	*Structure*	Substrate
CHQ chlorohydroquinone		peanut hay, seed, hulls, and vines soybean forage, hay and vines
2,4-DB glycine 4-(2,4-dichlorophenoxy)-butyryl glycine		goat milk, liver, and kidney

Table 2. Summary of characterized/identified residues in forage and hay of alfalfa treated with uniformly ring-labeled [^{14}C]2,4-DB at 1.1x.

Metabolite	0-day PHI Forage[a]		59-day PHI Forage[a]		59-day PHI Hay[a]	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified						
2,4-DB	98.43	71.45	56.91	2.34	48.47	7.96
2,4-DB methyl ester	0.62	0.45	--	--	--	--
2,4-D	0.52	0.38	3.20	0.13	0.36	0.06
2,4-D phenol	1.13	0.82	0.90	0.04	0.54	0.09
4-CPA	0.09	0.07	8.16	0.34	7.86	1.29
2,5-Cl-4-HPAA	--	--	--	--	1.01	0.17
2,5-Cl-4-HPBA	--	--	7.84	0.33	7.88	1.29
2,4-Cl-6-HPAA	--	--	--	--	2.63	0.43
2,4-Cl-6-HPBA	--	--	--	--	0.15	0.02
4-CP	--	--	--	--	1.66	0.27
3,5-Cl-2-HPBA/ 2,4-Cl-6-HPBA	--	--	5.92	0.25	8.95	1.47
4,6-DCR/ 2,3-Cl-4-HPBA/ 2,5-Cl-4-HPBA	--	--	0.41	0.02	--	--
Total identified	**100.79**	**73.17**	**83.34**	**3.45**	**79.51**	**13.05**
Total characterized/ identified	**<106.02**	**75.41**	**<85.91**	**<3.53**	**81.79**	**13.37**
Non-extractable	<1.0	0.01	<1.0	0.02	<1.0	0.11

[a] The formulated test material was applied as a single broadcast spray, at a field-equivalent rate of 1.65 lb ae/A (1.1x the maximum seasonal use rate) to alfalfa at the 3rd to 4th trifoliate leaf stage. Forage samples were harvested at posttreatment intervals (PHIs) of 0 and 59 days corresponding to 0-day PHI forage and 59-day PHI forage, respectively. A subsample of 59-day PHI forage was made into hay by drying in a tray in a greenhouse for 10 days (to 27% moisture).

Table 3. Summary of characterized/identified residues in matrices from peanuts following two applications of [^{14}C]2,4-DB at 1.1x.

Metabolite	Forage[a]		Hay[a]		Seed[a]		Hulls[a]		Vines	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Identified										
2,4-DB	50.05	21.91	54.86	130.75	--	--	--	--	1.43	0.05
2,4-DB butyl ester	0.19	0.08	0.11	0.26	--	--	--	--		--
2,4-DB ethyl ester	--	--	--	--	--	--	--	--	0.04	<0.01
2,4-DB methyl ester	0.38	0.17	0.07	0.17	--	--	--	--	--	--
2,4-D	1.76	0.77	3.02	7.20	--	--	--	--	--	--
2,4-D phenol	--	--	2.39	5.70	--	--	--	--	--	--
4-CPA	1.33	0.58	2.34	5.58	0.59	<0.01	--	--	3.68	0.13
4-CPA butyl ester	--	--	0.17	0.41	--	--	--	--	0.04	<0.01
CHQ	--	--	0.43	1.02	1.65	<0.01	3.28	<0.01	0.66	0.02
4,6-DCR	--	--	0.32	0.76	--	--	--	--	--	--
2,3-Cl-4-HPAA	0.34	0.15	--	--	1.96	<0.01	4.41	<0.01	1.27	0.05
2,3-Cl-4-HPBA	0.33	0.14	--	--	--	--	--	--	--	--
2,4-Cl-3-HPAA	2.74	1.20	3.01	7.17	--	--	--	--	2.81	0.10
2,4-Cl-5-HPAA	0.38	0.17	--	--	--	--	--	--	4.26	0.15
2,4-Cl-5-HPBA	--	--	1.30	3.10	--	--	--	--	0.37	<0.02
2,4-Cl-6-HPAA	5.36	2.35	4.19	9.99	1.85	<0.01	--	--	18.28	0.66
2,4-Cl-6-HPBA	--	--	--	--	--	--	--	--	0.11	<0.01
2,5-Cl-4-HPBA	1.37	0.60	0.11	0.26	--	--	--	--	--	--
2,3-Cl-4-HOH	2.29	1.00	1.97	4.70	--	--	--	--	9.92	0.36
2,4-Cl-6-HOH	--	--	--	--	--	--	--	--	3.32	<0.13
2,4-D/2,4-Cl-6-HOH	--	--	0.25	0.60	--	--	--	--	0.46	<0.02
2,4-D butyl ester/4-CPA butyl ester	--	--	0.11	0.26	--	--	--	--	--	--
2,4-D ethyl ester/ 2,4-DB methyl ester	0.91	0.40	0.13	0.31	--	--	--	--	--	--
4-CPA/2,4-Cl-6-HPAA	--	--	--	--	--	--	--	--	0.05	<0.01

(continued; footnotes follow)

Table 3 *(continued)*.

Metabolite	Forage[a]		Hay[a]		Seed[a]		Hulls[a]		Vines	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
2,3-Cl-4-HPBA/2,5-Cl-4-HPBA	0.07	0.03	2.86	6.82	--	--	--	--	--	--
2,4-Cl-6-HPBA/3,5-Cl-2-HPBA	0.80	0.35	--	--	--	--	--	--	--	--
2,4-D phenol/2,4-Cl-5-HPBA	1.91	0.84	--	--	--	--	--	--	--	--
2,5-Cl-4-HOH/2,4-Cl-3-HPAA	--	--	0.23	0.55	4.42	0.01	--	--	--	--
2,4-D phenol/2,4-Cl-6-HPBA/ 3,5-Cl-2-HPBA	--	--	--	--	--	--	--	--	2.95	0.11
2,4-Cl-5-HPAA/4-CPA/ 2,4-Cl-6-HPAA	--	--	--	--	--	--	--	--	0.61	0.02
2,3-Cl-4-HPBA/4-CP/ 2,5-Cl-4-HPBA	0.07	0.05	--	--	--	--	--	--	--	--
2,4-Cl-5-HPAA/4-CPA/ 2,4-Cl-6-HPAA	--	--	--	--	--	--	--	--	2.92	0.11
2,4-Cl-5-HPAA/4,6-DCR/ 4-CP/2,4-Cl-6-HPAA	2.21	0.97	--	--	--	--	--	--	--	--
2,4-Cl-5-HPBA/2,4-D phenol/ 4-CPA methyl ester	0.11	0.05	0.14	0.33	--	--	--	--	--	--
2,4-Cl-6-HPAA/4,6-DCR/ 2,4-Cl-5-HPAA	0.29	0.13	1.26	3.00	--	--	--	--	--	--
2,5-Cl-4-HPBA/2,4-D/ 2,3-Cl-4-HPBA	--	--	0.89	2.12	--	--	--	--	--	--
2,5-Cl-4-HPAA/2,4-Cl-3-HPAA/2,5 -Cl-4-HOH	--	--	--	--	--	--	--	--	1.06	0.04
2,4-Cl-3-HOH/2,3-Cl-4-HPAA/ 2,5-Cl-4-HPAA	--	--	0.54	1.29	--	--	--	--	--	--
2,5-Cl-4-HOH/2,4-Cl-3-HOH/ 2,5-Cl-4-HPAA/2,3-Cl-4-HPAA	--	--	0.22	0.52	--	--	--	--	--	--

(continued; footnotes follow)

Table 3 *(continued)*.

Metabolite	Forage[a]		Hay[a]		Seed[a]		Hulls[a]		Vines	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
2,4-Cl-6-HPBA/2,4-D phenol/ 3,5-Cl-4-HPBA/4-CPA methyl ester	--	--	0.13	0.31	--	--	--	--	--	--
2,4-Cl-5-HPBA/4,6-DCR/ 2,5-Cl-4-HPBA/4-CP	--	--	--	--	--	--	--	--	0.49	0.02
2,4-Cl-3-HOH/2,3-Cl-4-HPAA/ 2,5-Cl-4-HPAA/2,3-Cl-4-HOH	--	--	0.07	0.17	--	--	--	--	--	--
Total identified	**72.89**	**31.90**	**81.12**	**193.34**	**10.47**	**<0.02**	**7.69**	**<0.01**	**54.73**	**1.98**
Total characterized/identified	***72.89***	***31.90***	***81.12***	***193.34***	***63.47***	***0.10***	***97.69***	***0.10***	***54.73***	***1.98***
Non-extractable	7	3.06	10	23.83	--	--	--	--	49	1.77

[a] Two foliar applications of the formulated test substance were made at a field equivalent rate of 0.44 lb ae/A/application for a seasonal rate of 0.88 lb ae/A (1.1x the maximum seasonal application rate); the first application (Treatment I) was made 26 days after planting and the second application (Treatment II) was made 21 days after the first application. Following treatments, the plants were maintained outdoors until onset of cold weather (54 days after treatment II), when the plants were transferred and maintained in a greenhouse until harvest. Forage samples were harvested 0 days after treatment I (0-day PHI I) and 0 days after treatment II (0-day PHI II). A subsample of 0-day PHI II forage was dried to 15% moisture for 5 days on a plastic enclosed drying table in the greenhouse to make hay. Vine samples were cut at the soil level and peanuts were pulled from the soil at 148 days after treatment II (148-day PHI II). The peanuts were separated into seed and hulls

(continued; footnotes follow)

Table 4. Summary of identified and characterized residues in matrices from soybeans treated with uniformly ring-labeled [^{14}C]2,4-DB at 1.1x.

Metabolite	34-day PHI Forage[a]		34-day PHI Hay[a]		84-day PHI Seed[a]		84-day PHI Pods[a]		84-day PHI Vines[a]	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Identified										
2,4-DB	19.75	1.05	16.17	3.34	--	--	2.65	0.01	8.82	0.49
2,4-DB butyl ester	--	--	0.12	0.02	1.06	<0.01	0.78	<0.01	--	--
2,4-D	0.42	0.02	0.01	<0.01	--	--	--	--	3.43	0.19
2,4-D phenol	0.15	0.01	0.68	0.14	--	--	--	--	--	--
2,4-D ethyl ester	--	--	<0.03	<0.02	--	--	--	--	1.04	0.06
1,4-BQ	1.87	0.10	7.16	1.38	21.75	<0.01	25.26	0.05	25.10	1.62
4-CPA	0.25	0.01	0.19	0.04	--	--	--	--	0.23	0.01
4-CPA butyl ester	0.08	0.01	0.15	0.03	--	--	--	--	0.43	0.03
4-CP	--	--	<0.02	<0.01	--	--	--	--	--	--
CHQ	5.62	0.30	3.86	0.80	--	--	--	--	1.32	0.07
2,3-DCA	2.69	<0.15	1.65	0.34	--	--	--	--	5.37	0.30
4,6-DCR	3.07	0.16	2.88	0.60	--	--	--	--	5.68	0.32
2,3-Cl-4-HPAA	17.66	0.94	--	--	--	--	--	--	1.97	0.11
2,3-Cl-4-HPBA	--	--	0.66	0.14	--	--	--	--	0.19	0.01
2,4-Cl-3-HPAA	0.29	0.02	11.36	2.36	--	--	--	--	0.85	0.05
2,4-Cl-5-HPAA	--	--	0.56	0.12	--	--	--	--	1.74	0.10
2,4-D methyl ester	--	--	--	--	--	--	--	--	0.06	<0.01
2,4-Cl-5-HPBA	0.10	0.01	--	--	--	--	--	--	2.04	<0.12
2,4-Cl-6-HPAA	0.38	0.02	5.52	1.15	--	--	11.58	0.02	7.29	0.41
2,4-Cl-6-HPBA	--	--	--	--	--	--	--	--	1.77	0.10
2,5-Cl-4-HPAA	0.83	0.05	--	--	--	--	6.02	0.01	3.67	0.20
3,5-Cl-2-HPBA	0.48	0.03	--	--	--	--	--	--	--	--
2,3-Cl-4-HOH	--	--	0.15	0.03	--	--	--	--	0.29	0.02
2,4-Cl-6-HOH	--	--	0.85	0.18	--	--	--	--	0.58	0.03
2,4-D phenol/2,4-D	--	--	--	--	--	--	--	--	2.10	0.12

Metabolite	34-day PHI Forage[a]		34-day PHI Hay[a]		84-day PHI Seed[a]		84-day PHI Pods[a]		84-day PHI Vines[a]	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
4-CPA/2,4-Cl-6-HPAA	7.21	0.38	0.30	0.06	--	--	--	--	0.06	<0.01
4-CPA methyl ester/ 2,4-Cl-6-HPBA/3,5-Cl-2-HPBA	0.10	0.01	0.19	0.04	--	--	--	--	0.71	0.04
2,4-Cl-5-HPBA/ 2,4-D phenol	--	--	0.22	0.05	--	--	--	--	--	--
2,4-Cl-6-HPBA/ 3,5-Cl-2-HPBA	--	--	0.74	0.15	--	--	--	--	--	--
2,4-Cl-5-HPAA/4,6-DCR	--	--	0.17	0.03	--	--	--	--	--	--
2,5-Cl-4-HPBA/ 2,3-Cl-4-HPBA	1.51	0.08	0.61	0.13	--	--	--	--	--	--
2,5-Cl-4-HPBA/ 2,3-Cl-4-HPBA/2,4-D	--	--	0.07	0.01	--	--	--	--	--	--
2,3-Cl-4-HOH/2,4-Cl-3-HOH/2,3-Cl-4-HPAA	--	--	2.56	0.53	--	--	--	--	--	--
2,4-Cl-6-HOH/2,4-D	6.93	0.37	--	--	--	--	--	--	--	--
2,4-Cl-6-HOH/2,4-D/ 2,3-Cl-4-HPBA/2,5-Cl-4-HPBA	--	--	5.40	1.12	--	--	--	--	--	--
2,5-Cl-4-HOH/ 2,4-Cl-3-HPAA	--	--	<0.01	<0.01	--	--	--	--	--	--
Total identified	**69.39**	**3.70**	**62.28**	**12.93**	**22.81**	**0.02**	**46.29**	**0.09**	**74.74**	**4.17**
Total characterized/ identified	**79.04**	**4.21**	**82.77**	**17.02**	**75.99**	**0.05**	**71.96**	**0.14**	**91.00**	**5.08**
Non-extractable	8	0.43	--	--	10	<0.01	27	0.05	15	0.84

Metabolite	34-day PHI Forage[a]		34-day PHI Hay[a]		84-day PHI Seed[a]		84-day PHI Pods[a]		84-day PHI Vines[a]	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm

[a] The treatment solution was applied once to field-grown soybean plants at midbloom stage as a broadcast spray, at a field-equivalent rate of 0.25 lb ae/A (1.1x the maximum postemergence broadcast application rate). Following application of the test substance, plants were maintained outdoors until onset of cold weather, when the plants were transferred to a greenhouse until harvest. Forage samples were harvested at PHIs of 0 and 34 days, when the plants were 50-60% pod set. Half of the 34-day PHI forage sample was made into hay by drying it on a table until it reached 15% moisture. Mature plants were harvested at a PHI of 84 days and the pods, seeds, and vines were separated.

Ruminant and Poultry Metabolism Study

Separate goat and chicken metabolism studies were also performed with 2,4-DB in an attempt to elucidate the metabolic pathways in ruminants and poultry. These results from these studies are discussed below:

Ruminant: The ruminant metabolism studies with [^{14}C]2,4-DB were performed with dairy cows dosed at 18.9 ppm in the diet for three consecutive days. Based on current tolerances of 0.2 ppm on alfalfa, soybean, and peanuts, this dose corresponds to ca 41x the maximum theoretical dietary burden (the submitted plant metabolism study, however, suggests that the theoretical maximum dietary exposure may increase significantly upon receipt of outstanding field residue data which may result in the current metabolism study reflecting less than a 1x dosing rate). The registrant identified 53% of the TRR in kidney, 85% of the TRR in milk, and 62% of the TRR in liver. TRR in fat and muscle amounted to <0.006 ppm and the registrant did not attempt further identification/characterization of these residues. In kidney and liver, parent 2,4-DB and its 2,4-D phenol metabolite were the principle residues identified, while in milk a glycine conjugate of 2,4-DB was the only compound detected.

Based on the evidence presented, it is proposed that 2,4-DB undergoes conjugation in ruminant to form 2,4-DB glycine or undergoes dealkylation to produce 2,4-D and 2,4-D phenol.

The TRR and residue information for liver, kidney, milk, and other matrices is presented in Table 5, and the registrant's proposed metabolic pathway in ruminants is appended to this review as Attachment 1.

Poultry: The poultry metabolism study on [^{14}C]2,4-DB was performed with laying hens dosed at 25.2 ppm in the diet for three consecutive days. This corresponds to a dose of ca. 195x the maximum theoretical dietary burden based on current tolerances. The registrant identified 58% of the TRR in liver, 70% of the TRR in fat, and 86% of the TRR in egg yolk. In muscle and egg white, TRRs were less than 0.05 ppm and no further identification was attempted. In all matrices in which metabolites were identified, parent 2,4-DB comprised the only significant portion of TRR.

Based on the evidence presented, it is proposed that 2,4-DB is not readily metabolized by hen and is primarily incorporated into body fat and fatty components of egg yolk as bound or conjugated 2,4-DB. Dealkylation of 2,4-DB to 2,4-D and 2,4-D phenol is a minor metabolic pathway in poultry.

The TRR and residue information for liver, muscle, and egg matrices is presented in Table 5 and the registrant's proposed metabolic pathway in hens in appended to this review as Attachment 2.

cc: RF, SF, List A Rereg. F., Circ., DJM.
RDI: Pilot Team: 11/9/95;RPerfetti:11/9/95;EZager:11/13/95.

Table 5. Summary and Comparison of [^{14}C]2,4-DB Goat and Hen Metabolites in Liver, Kidney, Milk, Egg, Muscle, and Fat Matrices

Metabolite or Fraction	% TRR (ppm)									
	Liver		Kidney		Milk or Egg		Fat		Muscle	
	Goat	Hen	Goat	Hen[b]	Goat	Hen	Goat[b]	Hen	Goat	Hen
2,4-DB	22.6% (0.053)	54.0% (0.078)	10.2% (0.011)	N/A		86.3% (0.525)	--	70.0% (0.257)	NA	--
2,4-D	0.8% (0.002)	2.1% (0.003)	2.5% (0.003)	N/A				--	N/A	--
2,4-D Phenol	29.1% (0.068)	1.6% (0.002)	32.2% (0.034)	N/A				--	N/A	--
2,4-DB Glycine	9.1% (0.021)	--	8.5% (0.009)	N/A	85.0% (0.306)	--		--	N/A	--
Characterized	--	27.5% (0.039)				13.5% (0.082)		2.7% (0.010)		76.7% (0.035)
Unidentified	17.6%[a] (0.042)	3.6% (0.005)	24.6%[a] (0.025)		3.2% (0.011)	8.9% (0.054)		13.5% (0.050)		--
Non-extractable	1.5% (0.003)	3.0% (0.004)	4.9% (0.005)		4.0% (0.014)	6.3% (0.038)				15.7% (0.007)
TOTAL	80.7% (0.189)	91.8% (0.131)	89.9% (0.087)	N/A	92.2% (0.331)	115% (0.699)		86.2% (0.317)	N/A	0.042 (92.4%)

N/A Not analyzed
-- Not Detected
[a] Unidentified residues in each case were comprised of 3 unknowns, none of which exceeded 14% TRR or 0.020 ppm.
[b] Poultry kidney is not required to be analyzed per Agency Guidelines. Ruminant fat was not analyzed since TRR levels were low at 0.003 ppm.

ATTACHMENTS

Attachment 1

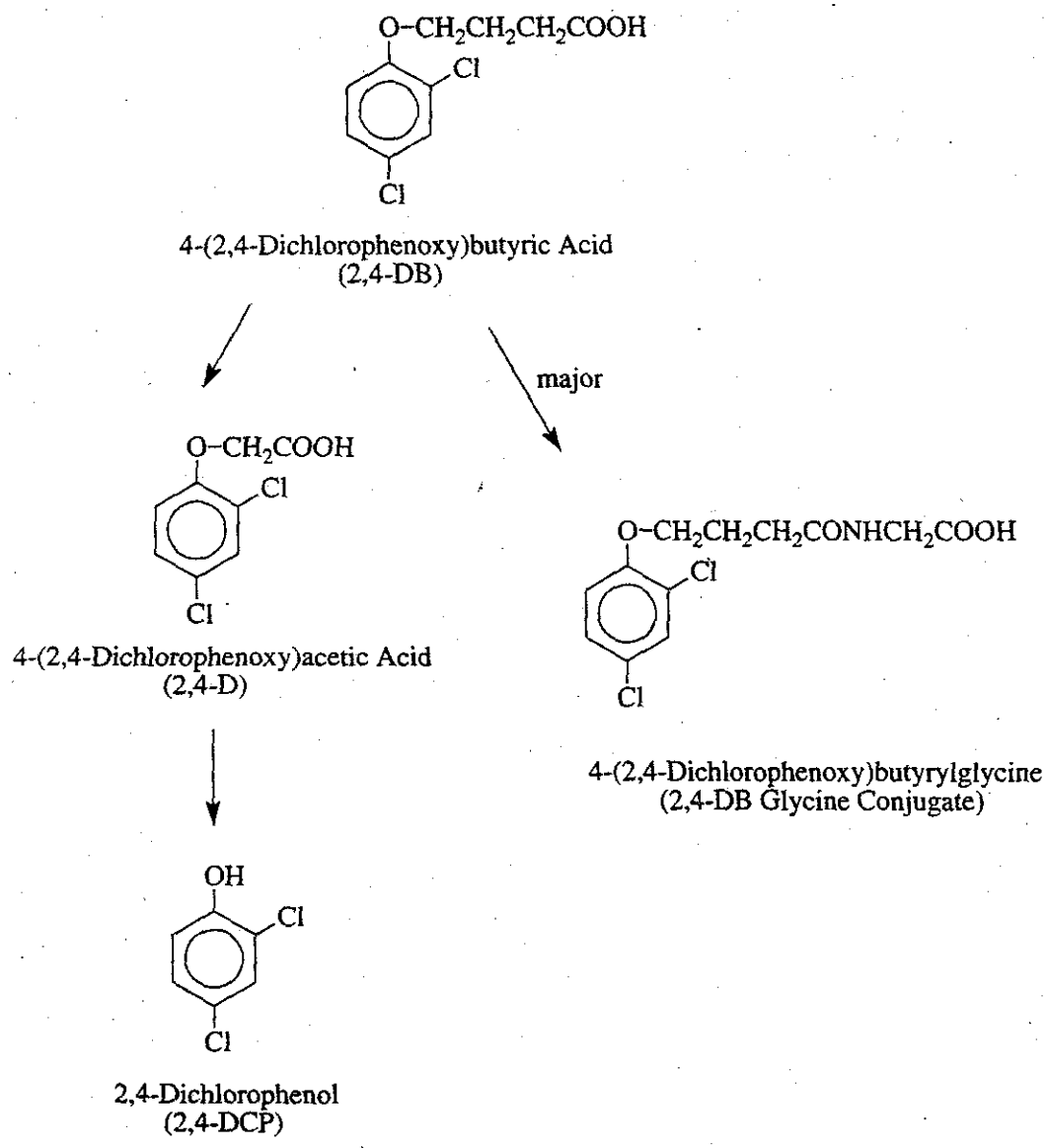


Figure 33. Proposed Metabolic Pathway of 2,4-DB in the Goat.

Attachment 2

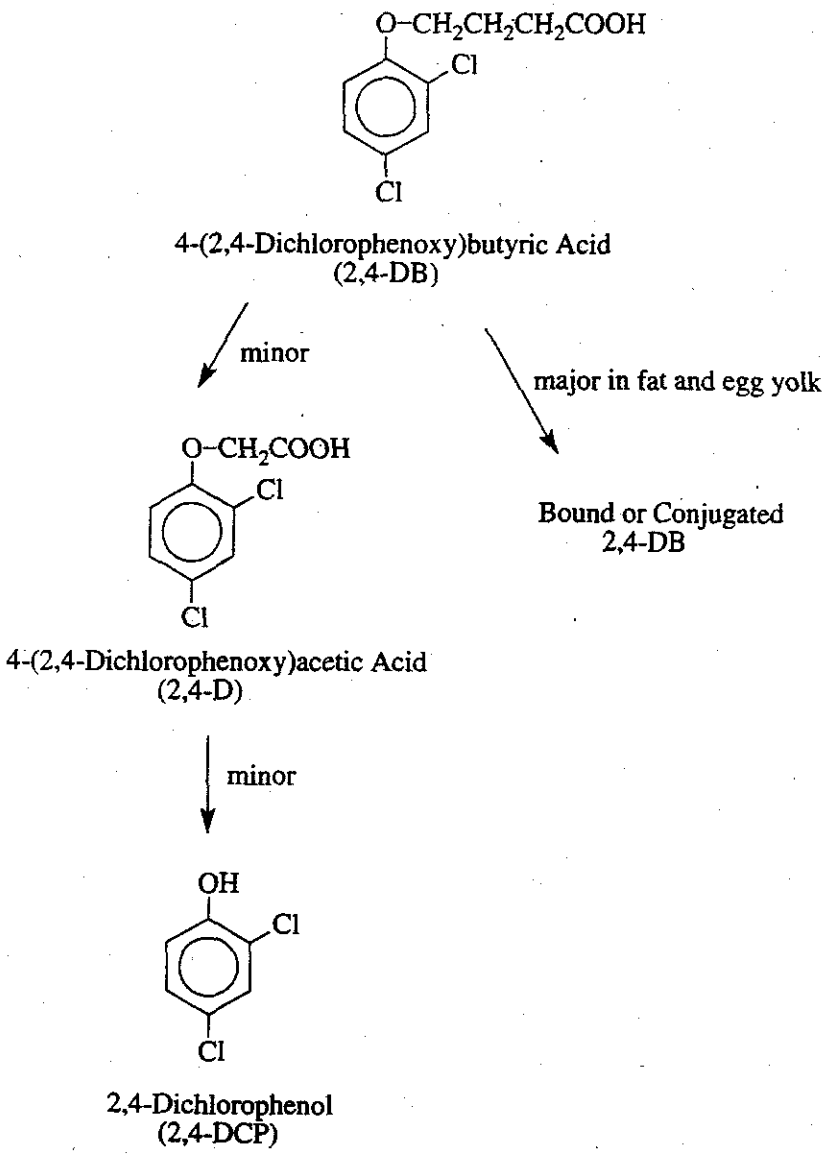


Figure 35. Proposed Metabolite Pathway of 2,4-DB in Poultry.

13544

030497

Chemical:	**4-(2,4-Dichlorophenoxy)butyric acid**
PC Code:	**030801**
HED File Code	**21400 MARC**
Memo Date:	**11/14/95**
File ID:	**DPD220888**
Accession Number:	**412-02-0011**

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